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Effects of fresh and aged vehicular particulate emissions on blood pressure in normal adult male rats

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Abstract Associations between exposure to fine particulate matter and blood pressure responses have been reported in epidemiological studies but findings have proven inconsistent. The objective of this study was to measure effects of primary and secondary components of traffic-derived fine particulate matter ($PM_{2.5}$) on blood pressure (BP). Sprague–Dawley rats were exposed to fresh primary vehicular particles (P), secondary organic aerosol (SOA), photochemically aged primary plus secondary organic aerosols (P+SOA) or filtered air for 5 h per day for three consecutive weeks. Particle concentration target was 50 μg/m^3 for all exposures. Blood pressure parameters were measured continuously using implanted transmitters. Systolic (SBP) and diastolic blood pressure (DBP), mean pressure, pulse pressure, and heart rate responses were assessed using mixed effects models. Exposure to P resulted in increased SBP (p=0.03) and DBP (p=0.05) that was sustained across weeks. SOA exposure resulted in increases in SBP (p=0.07) and DBP (p=0.01) on the first day with this effect decreasing significantly across exposure days (p<0.0001). P+SOA showed significant increases in SBP (p=0.002) and DBP (p<0.0001) across weeks with a magnitude of effect equaling the approximate average of the effect estimates of the P and SOA exposures. Double Sham exposures following SOA and P+SOA showed compensatory decreases in SBP and DBP. No exposure had a significant effect on heart rate. Primary and secondary traffic derived aerosols can substantially increase SBP and DBP, but these increases are lost with continued exposures. Compensatory BP responses resulting after exposure to secondary particles require further investigation to define BP control mechanisms.

Keywords Particulate matter · Blood pressure · Traffic emissions · Hypertension

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Introduction

Ambient fine particle matter ($PM_{2.5}$) is a complex mixture of primary particles emitted directly into the atmosphere by a variety of sources and secondary particle matter formed in the atmosphere from precursors emitted by sources. $PM_{2.5}$ concentration and composition vary spatially and temporally, with secondary components contributing 30–90 % of the fine particle mass (Bell et al. 2007, Hodan and Barnard 2004). Nevertheless, information regarding sources, formation, composition and toxicity are still not well understood (Kleindienst et al. 2010).

Exposure to particulate matter (PM) and associated increases in morbidity and mortality are documented in numerous epidemiological studies (Brook et al. 2010). More specifically, $PM_{2.5}$ exposure has been linked to adverse cardiovascular health outcomes, including the induction of cardiac arrhythmias (Peters et al. 2000, Anselme et al. 2007), myocardial infarction (D'Ippoliti et al. 2003), and coronary vasoconstriction (Bartoli et al. 2009a). Concentrated ambient particles (CAPs) have also been used in exposure studies to associate health effects with particle sources using factor analysis techniques, but do not differentiate between primary and secondary particles.

Surrogate markers of vehicular emissions such as black carbon (BC) have been used in exposure and epidemiological

studies to associate traffic-derived particles with health effects. BC results from the incomplete combustion of fossil fuels, primarily diesel fuel, and accounts for a small percentage of ambient fine particles (USEPA 2009). Increases in blood pressure have previously been linked to exposure to vehicular emissions (Bartoli et al. 2009b; Brook and Rajagopalan 2009), though mechanisms responsible for these increases continue to be speculative. Vehicular emissions account for a large portion of ambient fine particulate (USEPA 2009).

Although ambient $PM_{2.5}$ consists of both primary and secondary components, traditionally, only primary $PM_{2.5}$ has been used to assess the effects of source-specific $PM_{2.5}$ on health outcomes in exposure studies. Since these components have typically not been separated in exposure experiments, potential differences in toxicity have not been determined in epidemiological or toxicological studies.

The present study uses an exposure system designed to simulate atmospheric photochemistry and produce exposures comprised of primary and/or secondary particles from fleet vehicular emissions contained in the ventilation stack of an urban highway tunnel (Papapostolou et al. 2012). The test atmospheres generated for this study were not intended to fully simulate a real world exposure but instead allowed us to investigate the effects of different components of air pollution generated by mobile sources. The decision to focus on the effects of the particle fraction was based on previous toxicological and epidemiological studies that have found the particulate fraction of traffic emissions to be associated with adverse cardiovascular and respiratory health outcomes (Batalha et al. 2002, Laden et al. 2000, Peters et al. 2004, Schwartz 2001). The gaseous fraction of traffic derived air pollution (specifically carbon monoxide) has mainly been utilized as a marker of the exposure. Indeed, a number of recent studies show low levels of carbon monoxide (Wellenius et al. 2004, Dolinay et al. 2004, Kim et al. 2006, Morse and Choi 2008, Ryter et al. 2004) and nitric oxide (Gianetti et al. 2002, Sethi et al. 2008) to have anti-inflammatory, vasorelaxant, and other potentially beneficial effects. These exposures were used to investigate effects on blood pressure responses as well as respiratory outcomes which are analyzed in a companion paper (Diaz et al. 2012). This exposure system design allowed for the unique ability to restrict the exposure to specific types of fine particles while continuously monitoring blood pressure and pulse (heart rate) in order to gain insight on traffic-related particle sources, toxicity, and effects on cardiovascular health.

Methods

Exposure system

The exposure system used in these studies is described in detail in a companion paper (Papapostolou et al. 2012). Briefly, a sampling line was run from the plenum of a major traffic tunnel (located in the northeast region of the United States) through a size selective inlet that removed particles larger than 2.5 μm in aerodynamic diameter, to a photochemical reaction chamber. Plenum air was added continuously to the chamber, with a mean residence time of 4 h. The chamber was irradiated using 180 40 W ultraviolet lamps (Sylvania, Danvers MA, National Biological Corp., Beachwood OH). Ozone (O_3) was added to the chamber to titrate the NO in the exhaust and decrease the time required to generate stable secondary mass output. The resulting reaction mixture was drawn continuously through two denuders in-parallel which were placed between the photochemical chamber and the point of animal exposures to reduce the concentrations of primary and/or secondary gases from the chamber output. Individual species were reduced by 80–90 % depending on their diffusion coefficients (Papapostolou et al. 2012). Particle size distribution and mass concentrations were measured using a scanning mobility particle sizer (SMPS Model 3934, TSI Inc., Shoreview, MN), in conjunction with a condensation particle counter (CPC Model 3785, TSI Inc). Ozone was measured by UV photometry (Model 49 C, Thermo Scientific, Waltham, MA).

Exposure atmospheres

Four types of exposure atmospheres were generated during this study. These included:

Primary particles (P). Traffic-derived pollutants including gasoline and diesel engine emissions, as well as brake, tire, and road dust were drawn from the plenum of the highway tunnel through the exposure system with lights turned off. Although some background ambient PM was included, the aerosol was dominated by traffic-derived pollutants. Primary gases were removed prior to exposure using a non-selective denuder.

Secondary organic aerosol (SOA). Primary particles were removed from vehicular emissions using a HEPA filter. The primary gases were then introduced into the photochemical reaction chamber along with sufficient O_3 to titrate the NO. The mixture was then irradiated, forming secondary aerosol. The secondary particles were predominantly organic but also contained some secondary sulfate and ammonium nitrate. Secondary gases and unreacted primary gases were removed prior to exposure using a non-selective denuder.

Aged primary plus secondary organic aerosol (P+SOA). Traffic-derived pollutants including primary gases and primary particles smaller than 2.5 μm in aerodynamic diameter were diluted in the photochemical reaction chamber with clean air and sufficient O_3 to titrate the NO. The mixture was then irradiated, forming secondary

particles. Again, the secondary PM contained some sulfate and nitrate but was predominantly organic. Secondary and unreacted primary gases were removed prior to exposure using a non-selective denuder.

Filtered air control (Sham). For all filtered air exposures, including baseline measurements, ambient air was drawn through a cylinder containing potassium permanganate-coated aluminum to remove oxidizable gases, then through a cylinder of activated carbon to remove organic compounds and finally through an in-line Opticap filter (Millipore Corp., Billerica, MA) to remove particles, after which the air was used for rat exposures.

Animals

Normal adult male Sprague–Dawley rats (250–300 g) (Taconic Farms, Inc., Rensselaer, NY) were acquired, housed, and maintained in accordance with the National Institute of Health guidelines for the use and care of laboratory animals. DSI PCA-40 telemeters (Data Sciences International-Ponemah, St. Paul, MN) were implanted intraperitoneally by Taconic Farms Inc. with a catheter, equipped with a pressure transmitter, which was inserted into the descending aorta for measurement of blood pressure and pulse parameters.

Between exposures, animals were housed within a Thoren Maxi-Miser Caging System (Hazleton, PA) outfitted with a HEPA filtering unit and a charcoal filter which together filtered air to each individual cage within the system. Each cage also had a filter cover while animals were housed to prevent extraneous exposure from ambient air and provide protection during transport from the mobile laboratory to the exposure chambers. Individual exposure chambers made of clear polycarbonate were placed on a specially designed anodized table with privacy panels to provide a shielded space during exposures. Exposure chambers were cylindrical in shape (10 cm in diameter× 18 cm in length) with connections for delivery of air. Further details of the mobile laboratory and experimental set up may be found in Godleski et al. (2011), Diaz et al. (2011) and Diaz et al. (2012).

Experimental design

Four rats were exposed to a traffic-derived aerosol, and four rats were used as control animals simultaneously exposed to filtered air during each experiment. Animals were exposed 5 h per day Monday through Thursday over the course of 3 weeks. Particle mass concentrations were consistently maintained for exposures for all particle types. The day after the final day of exposure for the SOA and P+SOA atmospheres, both the aerosol exposed and filtered air control animals were exposed to filtered air only in a double Sham experiment.

Exposures were conducted in the individual chambers described above and placed on Data Science International (DSI) receivers from which blood pressure measures could be recorded continuously (Diaz et al. 2011). The flow through all individual exposure chambers was maintained at 1.5 L/min for the duration of each exposure. During all exposures, blood pressure and heart rate were measured continuously from the previously implanted telemeters. These transmitters send digital data through radio frequency signals to PhysioTel Receivers (DSI, St. Paul, MN) which convert this data into numbers via Data Science, Inc. (DSI, St. Paul, MN) Dataquest software ART 4.0.

Statistics

Five parameters were analyzed: diastolic blood pressure (DBP), systolic blood pressure (SBP), mean pressure (P), pulse pressure (PP), and heart rate (HR). The continuous data were reduced to 10-min averages which were plotted to show each animal's pattern of response throughout the exposure. For each aerosol atmosphere, blood pressure outcomes for exposed and control groups were plotted against time within chamber for each day of exposure using box plots with an added smoothing line representing the mean of each group. All graphical representations of the data were created using JMP 9.0 (SAS Institute Inc., Cary, NC) unless otherwise noted. For additional exploratory analyses, the 10-min averages were averaged over the 5-h exposure period to obtain daily blood pressure measurements for each animal. These were then plotted across weeks to examine group-specific trends in each response across multiple days of exposure.

For statistical modeling of trends within an exposure period, the 10-min averages of each response variable over the 5-h exposure period were considered as a functional curve defining the changes throughout each exposure. Generalized additive mixed models (GAMM) were used to estimate a mean difference curve between exposed and control groups for each atmosphere and each outcome, along with 95 % point-wise confidence intervals (Coull et al. 2011). These analyses were conducted using the gam function in the mgcv package in *R* (Wood 2006).

To analyze how exposure effects changed over multiple exposures (weeks), mixed effects models based on the 10-min averages of continuous blood pressure measurements were utilized. Analyses were performed using PROC MIXED in SAS 9.2 (SAS Institute Inc., Cary, NC). Separate mixed models were generated for each blood pressure parameter in each aerosol mixture to analyze differences between exposed and control groups. To determine the overall effect of exposure to each aerosol versus filtered air, exposure was included

in the models as a binary variable. Each model for a given blood pressure outcome specified three distinct differences between the exposed and unexposed groups: at baseline, during the course of the exposure (first day effect, linear change and quadratic change) and during the follow-up double sham exposure.

Based on the exploratory graphical data analyses described above, the models assumed that the trend of the differences between exposed and unexposed groups follow a quadratic function of day of exposure. The full model is:

$$Y_{ij} = \beta_0 + \beta_1 \exp * \text{baseline} + \beta_2 \exp * \text{week123} + \beta_3 \exp * \text{week123} * \exp \text{osure number} + \beta_4 \exp * \text{week123} * \exp \text{osure number}^2 + \beta_5 \exp * \text{double Sham} + b_{1i} + b_{2j} + \varepsilon_{ij}$$

where β_0 is the intercept; β_1 is an interaction term for exposure group by baseline to determine whether the exposure group was significantly different from the control group at baseline; β_2 is an interaction term for exposure group by week to reflect the effect of exposure on the first day of exposure; β_3 is an interaction term for exposure group by exposure number; β_4 is an interaction term for exposure group by a quadratic function for the number of exposure; β_5 is an interaction term for exposure group by the double Sham; b_{1i} is the random effect for animal; b_{2j} is the random effect for exposure number; and ε_{ij} is the residual error. Both date and rat were included as random effects within the model to account for daily variability in control animals as well as heterogeneity between rats. A first order autoregressive covariance structure within each exposure period within each animal, which assumes measurements taken on a subject close together in time are more correlated than those farther apart, was used to provide a better fit to the within-exposure correlation structure. For all models, statistical significance was established at $p<0.05$.

The plots of the P atmosphere indicated there was a constant effect of exposure across all weeks with little variability across days, therefore the model for the P atmosphere contained only two of the coefficients from the full model comparing baseline (β_1) and the effect of exposure across weeks (β_2).

Separate models were analyzed to determine whether a dose–response relationship existed between the measured blood pressure parameters and particle concentration, particle count, nitric oxide, or nitric oxides. Analyses were performed using PROC MIXED in SAS 9.2 (SAS Institute Inc., Cary, NC). Each exposure component above was used as a single coefficient to describe each blood pressure parameter in each scenario. An example is shown below:

$\gamma_{ij}=\beta_0+\beta_1 X_1+b_{1i}+b_{2i}+\varepsilon_{ij}$ where β_0 is the intercept; β_1 is the exposure component; b_{1i} is the random effect for rat; b_{2i} is the random effect for exposure number; and ε_{ij} is the residual error. A first order autoregressive covariance structure was used and statistical significance was established at $p<0.05$.

Results

Exposure characteristics

The total particle mass concentration of the exposures was designed to be consistent for all aerosol atmospheres. Particle mass concentrations were targeted to be approximately 50 μg/m^3 and measurements were obtained from the SMPS. Table 1 shows a summary of the average concentrations of particle mass (microgram per cubic meter), particle count (thousands of particles per cubic centimeter), average mass median mobility diameter (MMD) and count median mobility diameter (CMD) in nanometers (nm), and nitric oxide (NO), and nitrogen oxides (NOx) in parts per billion (ppb) for each aerosol exposure. Average carbon monoxide concentrations across all exposure atmospheres were approximately 0.6 parts per million. Ozone measurements at the point of exposure were below the limit of detection of the instrument. The average mass concentration for all three exposure atmospheres was 54.4 ± 12.6 μg/m^3. Particle counts in the primary exposure were two to three times higher than those in the SOA exposure. NO and NO_x were also considerably higher in the primary exposure.

Blood pressure

Five continuous parameters were analyzed: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean pressure (P), pulse pressure (PP), and heart rate (HR). Figure 1 illustrates the average net effect of SBP and DBP differences (exposed minus control) across the entire exposure period for each aerosol type. Baseline estimates for all exposure aerosol groups indicated there was no significant difference from the Sham control groups. Patterns indicate that there was a sustained response across the weeks for the primary aerosol (P) where all net effect averages for both SBP and DBP were positive for the entire length of exposure.

In contrast, effect sizes were greatest on the first day of exposure to SOA and quickly decreased over time for both SBP and DBP. Positive net effects were observed in the first 2 days of exposure only, dipping below zero by the end of the first week, and remaining negative for the rest of the exposure period. The double Sham shown at the end of the plot illustrates the dramatic decrease in blood pressure in the previously SOA-exposed group for both SBP and DBP.

P+SOA responses reflect both primary and secondary aerosol exposures, with initial increases in blood pressure that were attenuated over time, though they were sustained

Table 1 Average concentrations±standard deviation of exposure measures for each aerosol atmosphere

Average concentration±SD						
Exposure	Particle mass (μg/m^3)	Particle Count (thousands of particles/cm^3)	MMD (nm)	CMD (nm)	NO (ppb)	NO_x (ppb)
P	53.3±13.2	20.7±4.2	321.1±20.7	106.9±7.4	43.9±51.8	83.5±34.4
SOA	53.5±20.0	5.8±2.2	366.4±10.6	164.9±14.4	2.0±0.0	31.6±6.3
P+SOA	56.4±4.6	8.6±1.2	297.4±6.0	135±2.3	1.0±0.0	2.8±1.8

Shown above are particle mass (based on SMPS measurements) in μg/m^3, particle count in thousands of particles/cm^3, mass median diameter (MMD) in nanometers (nm), count median diameter (CMD) in nanometers (nm), nitric oxide (NO) in parts per billion (ppb), and nitrogen oxides (NO_x) in parts per billion (ppb). The P+SOA atmosphere, on average, was made up of 30 % aged primary particles and 70 % secondary organic aerosol.

for a longer period than effects observed in the SOA exposure. Positive net effects were seen in both SBP and DBP across the first 2 weeks, but by the end of the second week, this effect had decreased and crossed zero. The double Sham for P+SOA showed a decrease in SBP and DBP of the previously exposed group, but it was a less dramatic effect

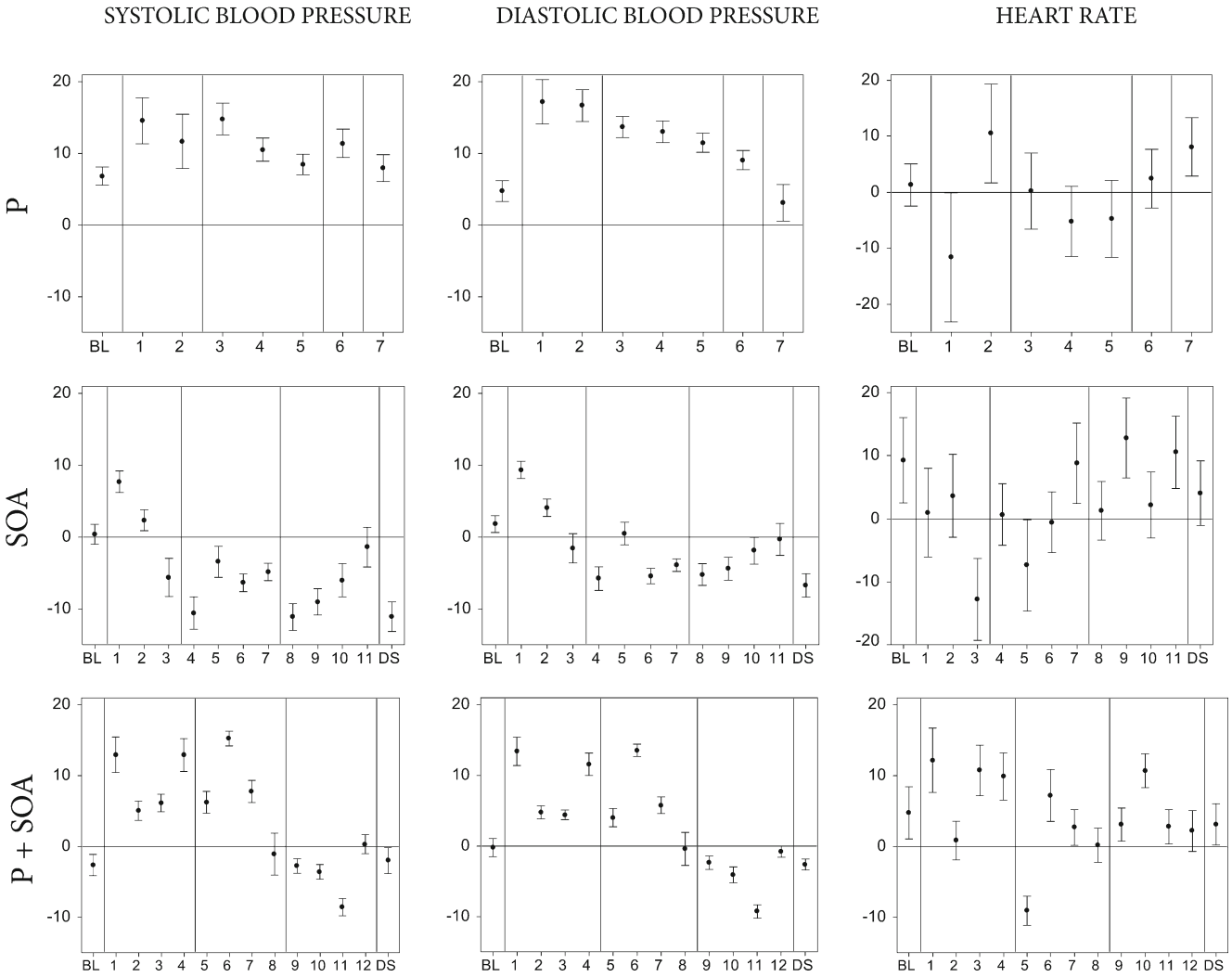

Fig. 1 Mean net effects±95 % confidence intervals of systolic and diastolic blood pressure and heart rate for each day of exposure. Vertical lines separate baseline (BL), each week of exposure and double-sham (DS). A double-sham exposure was not conducted for the primary aerosol

than observed in the SOA exposure. It is important to note that Fig. 1 showed no discernible patterns across days or weeks for effects on heart rate in any exposure.

The trends observed across the weeks of exposure were verified by statistical modeling and these results are presented in Table 2. For P aerosol exposures, the coefficient measuring effect of exposure by week was positive and significant with a beta coefficient of ~11 for both SBP and DBP, indicating a clinically relevant and sustained increase across weeks. This reflects the exploratory data plots shown in Fig. 1. The week effect was also significant for mean pressure with a beta coefficient of ~11. Pulse pressure was not significant. A double Sham exposure was not conducted after the P exposure. SOA showed substantial increases on the first day of exposure for SBP, DBP, and mean pressure. These increases were significant for DBP and mean pressure and nearly significant for SBP. The exploratory plots indicate a dramatic decrease after the first day of exposure for both SBP (β=−5.1, p<0.0001) and DBP (β=−4.4, p<0.0001) which is captured by the coefficient for exposure number. This decrease across exposure number followed a quadratic trend with a significance of <0.0001. Effect estimates for the double Sham exposure showed a strong decrease in SBP in the previously exposed group reflected by a beta coefficient of −9.9 (p=0.02) and a decrease in DBP of β=−5.9 (p=0.10). The P+SOA exposure showed a strong effect of week for both SBP (β=9.8, p=0.002) and DBP (β=9.8, p=<0.0001) with no significance for the effect of exposure number. The coefficient for quadratic trend showed minor decreases and significance for SBP (β=−0.12, p=0.05) but not for DBP (β=−0.1, p=0.09). The double Sham exposure also resulted in decreases of both SBP (β=−1.6, p=0.68) and DBP (β=−2.4, p=0.38) in previously exposed animals, though they were less dramatic than for SOA and not statistically significant. Overall, the magnitude of effect sizes in these models are similar to blood pressure changes seen in the data presented in Fig. 1.

Despite the relatively narrow range for exposure concentrations, particle count showed a significant dose–response relationship in the P aerosol with both SBP (β=0.46, p=0.02) and DBP (β=0.79, p=0.0002). Diastolic BP also showed a significant dose–response relationship with mass concentration in the P aerosol atmosphere, however, after removing the day with the highest mass concentration, this relationship disappeared, indicating a single high exposure could have been driving this effect. The dose–response relationship remained significant for particle count for the P aerosol exposure even after removing the same day with the highest concentration. No dose–response relationship existed for SOA or P+SOA with mass, particle count, or NO_x concentrations.

Figure 2 shows representative examples of the patterns seen during the 5-h exposure across all aerosol types. In these exploratory plots, mean SBP and DBP are plotted

Table 2 Statistical results of mixed effects models for all blood pressure parameters in each aerosol atmosphere

Aerosol atmosphere	Systolic pressure		Diastolic pressure		Heart rate		Pressure		Pulse pressure	
P[a]	Effect	p value	Effect	p value	Effect	p value	Effect	p value	Effect	p value
Baseline effect	6.4	0.21	4.6	0.45	1.4	0.91	4.9	0.33	0.96	0.85
Exposure effect	11.1	0.3*	11.8	0.5*	0.71	0.95	11.2	0.02*	−0.76	0.88
SOA[b]	Effect	p value	Effect	p value	Effect	p value	Effect	p value	Effect	p value
Baseline effect	−0.06	0.99	1.5	0.65	10.6	0.35	0.75	0.84	−2.2	0.17
First day effect	6.8	0.07	8.4	0.009*	4.2	0.68	7.5	0.03*	−1.7	0.26
Linear change	−5.15	<0.0001*	−4.4	<0.0001*	−2.4	0.37	−4.8	<0.0001*	−0.67	0.03*
Quadratic change	0.43	<0.0001*	0.37	<0.0001*	0.30	0.25	0.40	<0.0001*	0.06	0.04*
Double sham effect	−9.9	0.02*	−5.9	0.10	9.3	0.44	−8.0	0.05*	−3.5	0.04*
P+SOA[b]	Effect	p value	Effect	p value	Effect	p value	Effect	p value	Effect	p value
Baseline effect	−4.2	0.27	−1.4	0.62	−0.01	0.99	−2.7	0.37	−2.4	0.40
First day effect	9.8	0.003*	9.8	<0.0001*	10.0	0.40	9.6	0.0001*	0.43	0.88
Linear change	−0.01	0.99	−0.51	0.36	−2.7	0.31	−.16	0.80	0.43	0.18
Quadratic change	−0.13	0.05*	−0.08	0.09	0.18	0.45	−0.11	0.05*	−0.04	0.14
Double sham effect	−1.6	0.68	−2.4	0.38	−3.2	0.82	−1.9	0.53	0.46	0.87

Regression coefficients represent the exposed/sham difference for baseline and effect of week for the primary atmosphere. For the SOA and P+SOA atmospheres, regression coefficients represent the exposed/sham difference for baseline and effect of week for the exposed/sham difference with increasing exposure number, with a quadratic term for increasing exposure number and double sham. The double sham exposure was not conducted for the primary atmosphere. Statistical significance was established at p<0.05

*Statistical significance at p<0.05

[a] Model assumes constant effect across weeks

[b] Model assumes quadratic trend across exposure days

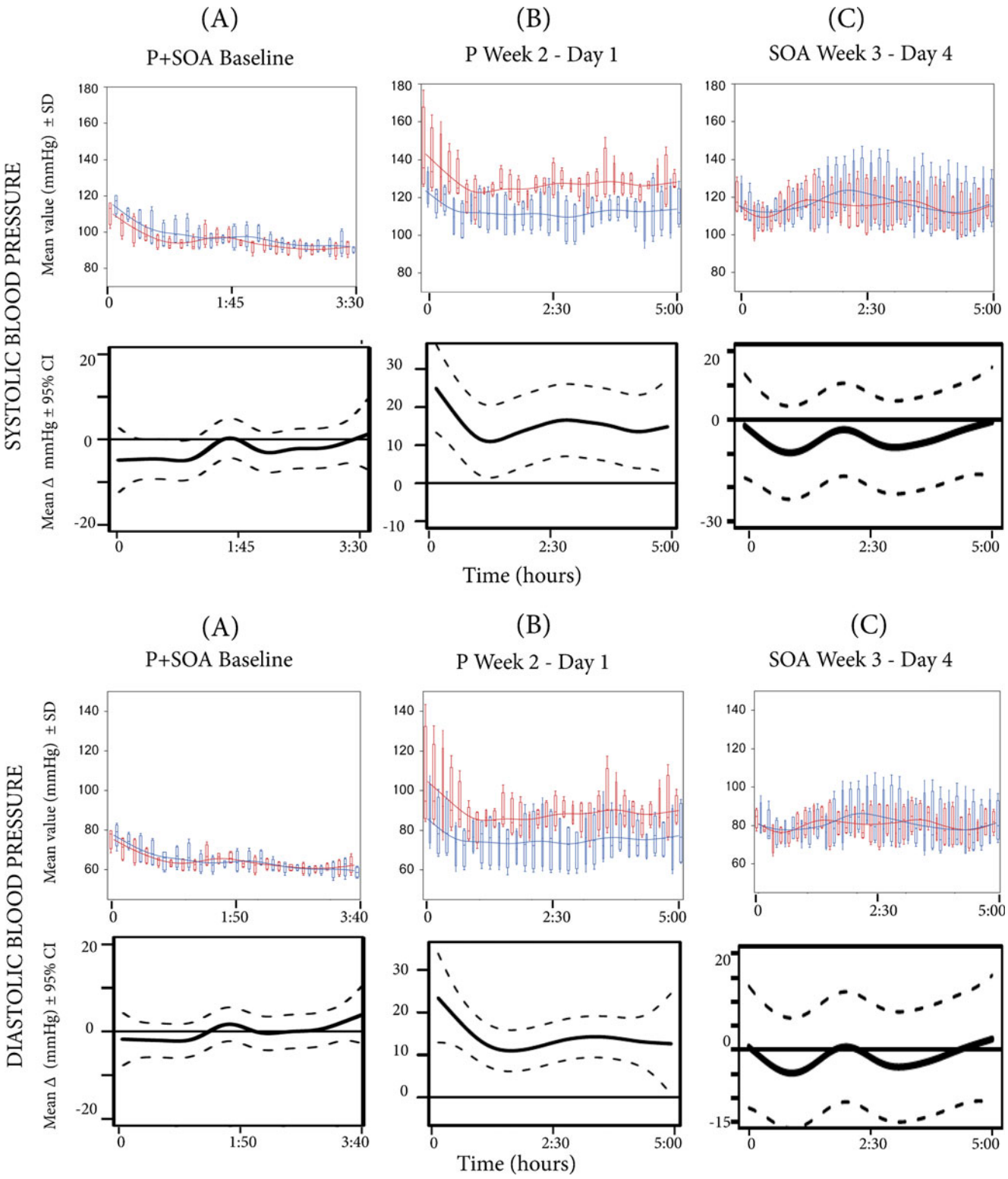

Fig. 2 Representative examples of daily trends showing consistency of data across all aerosol exposures. Mean SBP and DBP for exposed (*red*) and control (*blue*) groups over time with corresponding GAMM fit model showing net effects. **a** Baseline SBP and DBP for P+SOA exposure. No difference at baseline between exposed and control groups. **b** Large BP difference—week 2 day 1 for primary shown here. Approximately 20 mmHg between exposed and controls across 5-h exposure. **c** No difference—week 3 day 4 of SOA shows no difference between groups across the day of exposure. Similar trends seen for all aerosols where in general, effects on blood pressure remained constant within the 5-h exposure

against time in hours for the specified day of exposure by exposed (red) and control (blue) groups. Also shown are the corresponding GAMM fit net effect models showing mean response with 95 % confidence intervals. Baseline SBP and DBP are depicted for the P+SOA exposure in Fig. 2a, indicating there were no differences between groups prior to exposure. Figure 2b gives an example of large differences observed between exposed and control groups, showing both SBP and DBP, on the first day of week two in the P exposure. This difference was maintained across the entire day of exposure. In an example of an exposure day in which there was no effect, Fig. 2c presents the fourth day of week three for SOA. This shows that both SBP and DBP do not vary dramatically within a day and exemplifies the consistency of the data across time. Figure 3 illustrates the double Sham exposures over the 5-h exposure period where both the previously exposed and control groups received filtered air. The SOA double Sham showed that the SBP and DBP of both groups began the exposure at approximately the same level. The previously exposed group then experienced a dramatic decrease in pressure of nearly 15 mmHg below that of the control group and maintained this difference throughout the day.

This experiment was repeated after the P+SOA exposure. While the magnitude of difference was smaller, only approximately 5 mmHg difference, the trend was similar. The control and previously exposed groups began the exposure with approximately the same level of blood pressure. The previously exposed group then showed a decrease in SBP and DBP of nearly 20 mmHg by the end of the first hour of exposure. In this experiment, the control group also showed a decrease in pressure of about 15 mmHg, which resulted in a difference of 5 mmHg that was maintained throughout the day.

Variation in response across exposure scenarios was evaluated using Student's unpaired *t* tests, which indicated statistically significant differences between P vs. SOA and SOA vs. P+SOA (both $p<0.01$), but not statistically significant different for P vs. P+SOA for SBP. Trends for DBP were the same for P vs. SOA ($p<0.001$), but different for P vs. P+SOA ($p<0.05$) and SOA vs. P+SOA ($p=0.06$). These statistical tests support the net effect trends observed between exposure atmospheres presented in Fig. 1.

Discussion

This study allowed for the unique ability to compare health effects of both primary and secondary source-specific particles through the use of an exposure system designed to simulate atmospheric reactions. The use of aerosols from this exposure generation system for animal exposures provided a method for reproducible toxicological measures while serving as a model of traffic-related air pollution. Populations that reside in urban areas, where traffic emissions are a major source of air pollution (American Lung Association, 2011) could be susceptible to similar changes in blood pressure. The effectiveness of the exposure generation system along with the observation of significant health effects establishes an exposure model upon which future mechanistic studies in animals and humans may be based.

Our results suggest that exposure to relatively low concentrations of both primary and secondary traffic-derived fine particles, cause significant increases in both systolic and diastolic blood pressure that could be sustained or attenuated over time depending on the composition of the fine particles. Initial increases of as high as 15 mmHg in diastolic blood pressure were recorded for the P exposure. This is a greater blood pressure change than has previously been reported in the literature in animal or human studies for similar or substantially higher $PM_{2.5}$ concentrations (Urch et al. 2005, Bartoli et al. 2009b). SOA showed similar increases in both systolic and diastolic blood pressures with initial increases of approximately 10 mmHg. The P+SOA exposure combining aged primary and secondary organic particles, resulted in initial increases on average of approximately 12 mmHg in systolic and diastolic blood pressure which is an intermediate response between the responses to P and SOA particles separately.

Statistical results suggest that the primary particle mixture could be driving the elevation of blood pressure. Primary aerosol exposure showed a sustained effect across the weeks of exposure, which was not seen for SOA. In the SOA exposures, large effects were seen in the first day and then decreased dramatically, indicating potential evidence of a biological compensatory response. When the primary and secondary aerosols were combined, the effect was sustained for a longer period than SOA alone but eventually attenuated as time progressed. Results of a study conducted by Urch et al. (2005) showed a significant increase in DBP of 6 mmHg in healthy humans exposed to concentrated ambient particles plus ozone for a 2-h period. Further analyses indicated a strong association between these changes in DBP and the concentration of organic carbon in the exposure. While the exposure in Urch et al. (2005) is not identical to that of the current study, it does show a response in humans after a high but environmentally relevant exposure to $PM_{2.5}$. The observation of these effects in humans after a short exposure is consistent with the increases in BP observed in the current study.

The toxicological differences in blood pressure response to these atmospheres may have varying implications. The P atmosphere resulted in an increase in both SBP and DBP which was maintained for the duration of the exposure. This sustained effect points to the possible development of long-term adverse health outcomes such as hypertension and

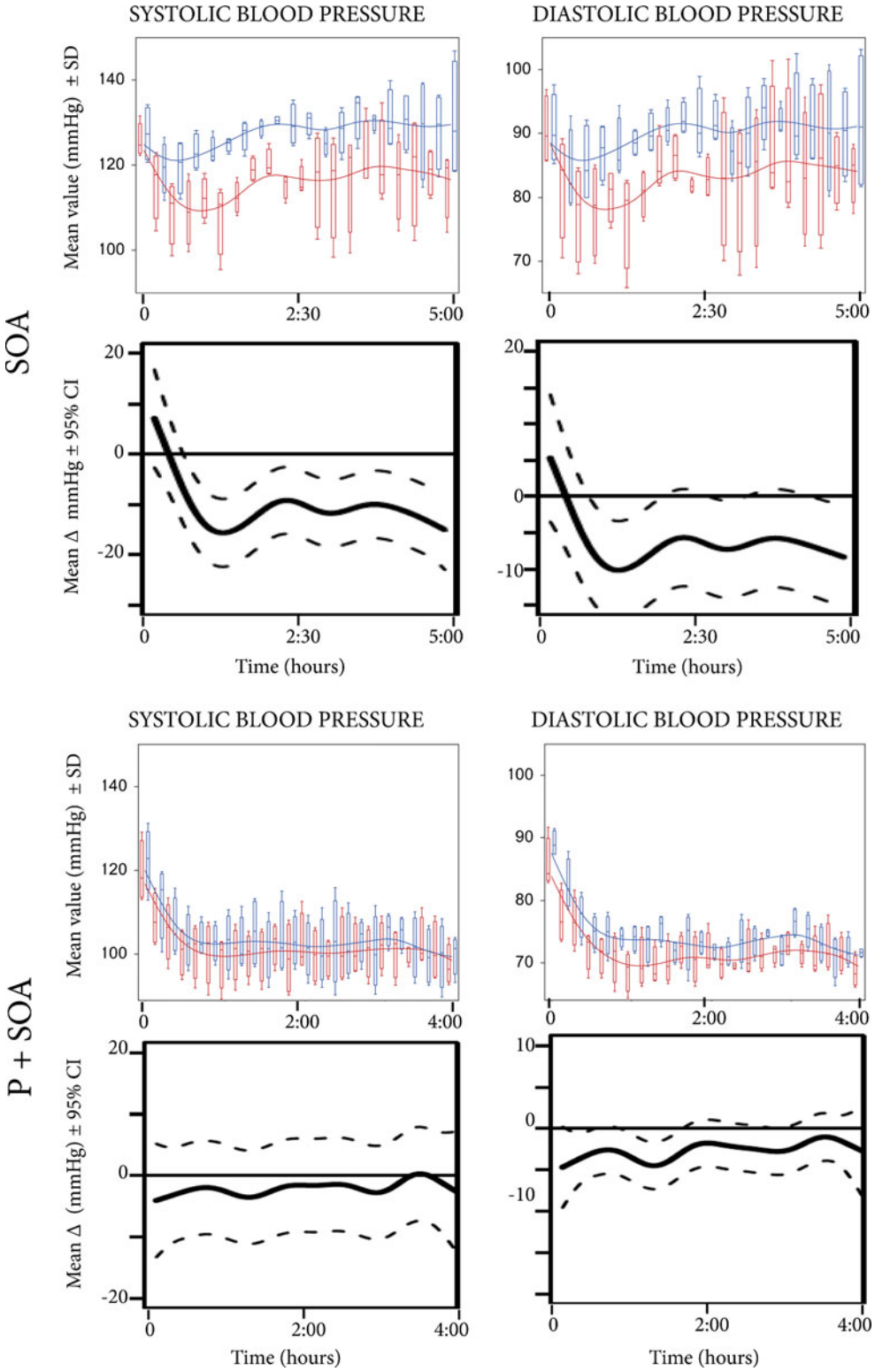

Fig. 3 Changes in SBP and DBP over time for SOA and P+SOA double Sham exposure. Mean SBP and DBP for exposed (*red*) and control (*blue*) groups over time with corresponding GAMM fit model showing net effects. A significant decrease in BP is seen in the SOA exposure showing a potential compensatory response. Decreases in the P+SOA exposure are not as dramatic but are consistent with trends seen in the SOA exposure

atherosclerosis, diseases that develop after repeated biological insult (Araujo and Nel 2009). Alternatively, the initial surge in blood pressure in the SOA atmosphere, which quickly attenuates, may have susceptibility implications for populations at risk of stroke or myocardial infarction (Franklin et al. 2007). Although no human exposures occur solely to primary or secondary traffic derived particles, the P+SOA atmosphere has more features of a "real world" exposure. This atmosphere showed an initial increase which was sustained for 2 weeks but eventually attenuated which points towards an emphasis on long-term adverse health outcomes as reflected by the P atmosphere. However, in environments where secondary

organic aerosol may predominate, such as in the summer months when temperatures and stronger UV light could promote increased secondary reactions, surges in blood pressure leading to acute health effects could be more of a concern.

In a study conducted by Delfino et al., 2010, elderly human subjects with coronary artery disease were exposed to an average $PM_{2.5}$ concentration of 21 μg/m^3 over 5 days. This exposure resulted in increases of SBP and DBP of 8.2 and 5.8 mmHg, respectively. The strongest associations in that study were found between blood pressure and organic carbon with primary organic carbon more strongly associated than secondary organic carbon. This study took place in Los Angeles, California, where emission inventories identify traffic as a major contributor to primary organic carbon, fine, and ultrafine particles (Sioutas et al. 2005, Hitchins et al. 2000, Zhu et al. 2002). Our study clearly supports the finding that primary traffic particles have a larger impact on elevation of blood pressure than secondary particles. Our exposure model thus provides an ideal system to further explore similar relationships found in human studies.

Evidence of a dose–response relationship in the primary aerosol exposure is shown with particle count for both systolic and diastolic blood pressure. The P atmosphere consisted of 100 % fresh primary particles, the P+SOA atmosphere consisted of 30 % aged primary particles and the SOA atmosphere had no primary particles. These percentages are consistent with primary particles being a major contributor to biological response, where a sustained effect was seen across weeks in the primary exposure, and only partially so for P+SOA. Primary aerosol showed the greatest amount of variability in exposure, the highest particle count and the lowest count median diameter. Particle count is typically dominated by the smallest particles, namely ultrafine particles (Hagler et al. 2009). Further analyses of the composition of these exposures are necessary to determine how that relates to sustained increases in blood pressure.

Traffic-derived ultrafine particles have been implicated in processes of oxidative stress and systemic inflammation (Mills et al. 2009). This might help to explain the sustained increases in blood pressure in the primary traffic aerosol exposure. Respiratory changes in the companion paper by Diaz et al. 2012 indicate the primary exposure resulted in a rapid shallow breathing pattern. However, since hyperventilation tends to result in vasodilation ultimately leading to a reduction in blood pressure, these results cannot explain the sustained effect of exposure to primary particles (Olschewski et al., 2001, Fineman et al., 1993). The SOA atmosphere resulted in decreased respiratory frequency, tidal volume, minute ventilation and flow, which suggested these animals were hypoventilating in response to the exposure. Literature is limited that relates such respiratory patterns and effects on blood pressure.

Sleep apnea studies examining effects of a repetitive partial or complete closing of the pharynx during sleep have shown these episodes result in "bursts" of sympathetic activity which rapidly increase blood pressure and heart rate (Silverberg et al. 2002, Kaneko et al. 2003). While these examples do not relate traffic exposure to breath holding, it does give evidence of a spike in blood pressure in response to these brief hypoxic events. One might attempt to explain the large initial increase in SBP and DBP in the SOA atmosphere as a result of the animals holding their breath however these increases were not accompanied by increases in heart rate.

Alternatively, a sustained controlled decrease in respiration could result in vasodilation as evidenced in studies that have looked at slow breathing exercises as a method for combating certain types of blood pressure increases (Madsen et al. 2008, Anderson et al. 2010). This might also help to explain the compensatory decrease seen in the SOA double sham experiments however, results for the P+SOA atmosphere showed increases in respiratory frequency. The P+SOA double sham experiment showed similar trends to that of the SOA double sham though not as drastic a response. The SOA and P+SOA atmospheres show similar response trends to the double sham experiments but dissimilar effects in respiratory outcomes. Therefore, respiratory changes cannot entirely explain the blood pressure response. As a whole, respiratory effects including pulmonary inflammation may contribute to the changes in blood pressure seen in these studies but do not offer a clear explanation of these relationships. The associations are complicated at best and indicate an alternative mechanism or mechanisms are involved.

Furthermore, the blood pressure changes cannot be explained by a simple stress response since changes in heart rate, a major indicator of sympathetic nervous system induction, were not significant in any exposure. Not only were effects attenuated over time, but when animals that had previously been exposed to particles were subsequently exposed to filtered air in the double Sham experiments, these animals subconsciously lowered their blood pressure (by approximately 10 mmHg in the SOA group and by about 5 mmHg in the P+SOA group) in response to an expected exposure. This effect was statistically significant for SOA. Mechanisms underlying this phenomenon have yet to be elucidated.

Two factors that could potentially contribute to subconscious decreases in blood pressure are changes to the sensitivity of the baroreceptor reflex (Bartoli et al. 2009b) or epigenetic modifications to the promoter regions of genes involved in vasodilatory/vasoconstrictive processes. The carotid baroreflex responds to a series of stretch stimuli from distention of the vasculature, triggered by increases in blood pressure or intravascular volume, that result in activation of

the parasympathetic nervous system (Taylor and Bisognano 2010). This leads to a reduction in cardiac contractility, increased dilatation of the peripheral vasculature along with decreased heart rate, which ultimately causes a decrease in blood pressure (Filippone and Bisognano 2007).

These decreases in blood pressure in our study cannot be attributed to a vasodilatory effect of NO or CO, as levels of NO were in the range of parts per billion and levels of CO averaged 0.6 parts per million for all aerosol exposures, whereas protective effects tend to be seen in the range of 50 to 500 parts per million (Otterbein et al. 1999, Ryter et al. 2004, Gianetti et al. 2002). The presence of nitrates within the aerosol mixture cannot be fully addressed in this paper. Organic nitrates may act as vasodilators, producing nitric oxide as a metabolic byproduct (Cohn et al. 2011), and therefore might contribute to the sharp decrease in blood pressure seen in the SOA and P+SOA exposures. However, this mechanism cannot completely explain the observed decreases, since the double Sham exposure did not contain nitrates but still showed a compensatory effect in the previously exposed group.

An important distinction between aerosol exposures exists within P+SOA since it is not a simple combination of primary and secondary aerosol as the name implies. During the exposures, a small concentration of ozone is continuously added to the chamber in order to titrate nitric oxide. In addition, substantial concentrations of ozone are formed during irradiation of the chamber. In the P+SOA exposure, ozone may react with organic compounds on the surface of the primary particles to change their composition, reactivity or potential for photochemical activation. This is in contrast to the primary particles in the P exposure, which are fresh and not chemically altered.

This study has some limitations. While the number of observations per animal on average was approximately 1200, the number of animals per group was a total of four. However, biological effects resulting from such a small group of animals at such a low level of exposure for a toxicological study are remarkable, since humans may be acutely exposed to comparable concentrations near highways and are likely to be exposed to similar concentrations for longer durations over a lifetime.

Conclusions

Significant changes in blood pressure are demonstrated here as a result of exposure from modest yet stable concentrations of pollutants. Sustained increases in blood pressure were observed and were maintained not only across entire days of exposure but over weeks of exposure, with the strongest effects seen in the primary aerosol atmosphere. In addition, an unexpected but highly significant compensatory response causing a dramatic decrease in both systolic and diastolic blood pressures was also seen after previously exposed animals were exposed to filtered air. This suggests a biological protective effect with repeated exposures that cannot be explained by simple autonomic nervous system activation. These results confirm the adverse health effects associated with inhalation of fine particles and give insight into a potential biological adaptation to maintain blood pressure in response to repeated and anticipated pollutant exposure. This study was able to successfully stratify the particulate exposure by primary and secondary components to allow for comparison of toxicity profiles and established a model for future studies relating blood pressure and traffic-related air pollution.

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